

CLAIMS

1. Process of creating at least one recombinant polynucleotide sequence characterized in that it comprises a step of oriented ligation of fragments derived from a bank of at least two polynucleotide sequences.

2. Process according to claim 1, wherein it comprises the following steps:

- a) fragmentation of a bank of polynucleotide sequences,
- b) denaturation of the fragments thus obtained,
- c) hybridization of fragments obtained in step (b) with one or several assembly matrix (matrices),
- d) oriented ligation of said fragments to obtain at least one recombinant polynucleotide sequence.

3. Process according to claim 2, wherein it comprises after step (d):

e) the selection of recombinant polynucleotide sequences offering advantageous characteristics compared to corresponding characteristics of one or several reference sequences.

4. Process according to claims 1 to 3, wherein the bank of polynucleotide sequences contains double-stranded polynucleotide sequences.

5. Process according to claims 1 to 3, wherein the bank of polynucleotide sequences contains single-stranded polynucleotide sequences.

6. Process according to claims 2 to 5, wherein at least one assembly matrix is double-stranded and it is first denatured to be added in single-stranded form at step (c).

7. Process according to claims 2 to 5, wherein at least one assembly matrix is single-stranded.

8. Process according to claims 2 to 7, wherein it comprises, at the end of step (d), at least one repetition of steps (a), (b), (c), and (d).

9. Process according to claims 2 to 7, wherein it comprises, at the end of step (d), at least one repetition of steps (b), (c), and (d).

10. Process according to claims 3 to 7, where it comprises, at the end of step (e), the choice of at least one recombinant polynucleotide sequence to perform at least one repetition of steps (a), (b), (c), (d), and (e).

11. Process according to one of claims 2 to 10, wherein it comprises the separation of recombinant polynucleotide sequences from the assembly matrix (matrices) before step (e).

12. Process according to any one of claims 2 to 11, wherein it comprises the amplification of recombinant polynucleotide sequences before step (e).

13. Process according to any one of claims 2 to 12, wherein it comprises, before step (e), the cloning of recombinant polynucleotide sequences, optionally after separation of recombinant strands from the matrix (matrices).

14. Process according to any one of claims 2 to 13, wherein the ends of the fragments produced at step (a) are such that there can be hybridization adjacent to these ends on at least one assembly matrix at step (c) and ligation of these fragments with one another at step (d).

15. Process according to any one of claims 2 to 14, wherein steps (c) and (d) are performed simultaneously.

16. Process according to any one of claims 2 to 15, wherein step (a) consists in subjecting the polynucleotide sequences of the initial bank to hydrolysis by the action of one or several restriction enzymes.

17. Process according to any one of claims 2 to 15, wherein the random fragmentation of polynucleotide sequences at step (a) is performed by any known enzymatic or mechanical method.

18. Process according to any one of claims 2 to 13 and 15 to 17, wherein enzymes able to recognize and degrade and/or cut in a specific manner the nonhybridized ends of fragments when said ends cover other hybridized fragments on the same matrix are added at step (c) and/or at step (d).

19. Process according to claim 18, wherein the enzyme Flap endonuclease is added at step (c) and/or at step (d).

20. Process according to any one of claims 2 to 15, wherein at least one specific, single-stranded exonuclease able to recognize and degrade in a specific manner the nonhybridized ends of fragments when said ends cover other hybridized fragments on the same matrix is added at step (c) and/or (d).

21. Process according to any one of the preceding claims, wherein a preferably thermostable ligase that is active at high temperature is used at step (d).

22. Process according to claims 18 and 19, wherein the endonucleases able to recognize and degrade and/or cut in a specific manner the nonhybridized ends of fragments added at step (c) and/or at step (d) have the same characteristics of thermoresistance and high-temperature activity as the ligase used at step (d).

23. Process according to claim 20, wherein the exonucleases able to recognize and degrade in a specific manner the nonhybridized ends of fragments added at step (c) and/or at step (d) have the same characteristics of thermoresistance and high-temperature activity as the ligase used at step (d).

24. Process according to any one of the preceding claims, wherein the initial bank of polynucleotide sequences is produced from a wild gene by successive steps of controlled mutagenesis, by error prone PCR, by random

chemical mutagenesis, by *in vivo* random mutagenesis, or by combining genes of near or distinct families within the same species or from different species so as to make available a variety of polynucleotide sequences in the initial bank.

25. Process according to any one of claims 2 to 23, wherein the initial bank of polynucleotide sequences consists of synthetic sequences that will be fragmented at step (a) or that can constitute the fragments of step (a).

26. Process according to any one of claims 2 to 16 and 18 to 25, wherein step (a) consists in subjecting the initial bank to hydrolysis by the action of restriction enzymes having a large number of cutting sites on the polynucleotide sequences of the initial bank, or in combining several restriction enzymes.

27. Process according to any one of claims 2 to 15 and 17 to 25, wherein step (a) consists of a random treatment with DNAase I from an initial bank of polynucleotide sequences.

28. Process according to any one of claims 2 to 15 and 17 to 27, wherein fragments produced by a random treatment are used as matrices for one another, for hybridization during step (c) or during the reaction of steps (c) and (d) simultaneously.

29. Process according to claims 2 to 16 and 18 to 26, wherein fragments obtained at step (a) by a treatment with restriction enzymes are used as matrices for one another, for hybridization during step (c) or during the reaction of steps (c) and (d) simultaneously.

30. Process according to claims 2 to 15 and 18 to 26, wherein the fragments of step (a) are obtained by amplification reactions performed on polynucleotide sequences of the initial bank using initiated oligonucleotides, making it possible to produce fragments having sequences in

common, said fragments acting as an assembly matrix for one another at step (b) or at step (c).

31. Process according to any one of the preceding claims, wherein the initial bank is fragmented into n fragments at step (a), n being greater than or equal to 3.

32. Process according to any one of the preceding claims, wherein besides the matrix, oligonucleotides of varying length, single- or double-stranded, are added at step (b) or (c).

33. Process according to any one of the preceding claims, wherein, before step (e), the recombinant polynucleotide sequences are separated from the assembly matrix thanks to a marker present on the assembly matrix or on the recombinant polynucleotide sequences.

34. Process according to any one of the preceding claims, wherein the recombinant polynucleotide sequences obtained at step (d) and optionally cloned are used by any appropriate means to select the recombinant polynucleotide sequences or the clones offering advantageous characteristics compared with corresponding characteristics of reference sequences.

35. Process according to claim 34, wherein the screening is performed by *in vitro* expression of recombinant polynucleotide sequences.

36. Process according to any one of the preceding claims, wherein the initial bank of polynucleotide sequences consists of one or several restricted banks prepared by a process according to any one of claims 1 to 35, optionally mixed with other polynucleotide sequences.

37. A recombinant polynucleotide sequence offering one or several advantageous characteristics compared to corresponding characteristics of reference sequences, obtained and selected by a process according to any one of claims 1 to 36, said sequence having a size greater than 1.5 Kpb.

38. A vector containing a polynucleotide sequence according to claim 37.
39. A cellular host transformed by a recombinant polynucleotide sequence according to claim 37 or by a vector according to claim 38.
40. A protein coded by a recombinant polynucleotide sequence according to claim 37.
41. A bank consisting of recombinant polynucleotide sequences according to claim 37, or of a vector according to claim 38, or of cellular hosts according to claim 39, or of proteins according to claim 40.